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Hormones and Sleep

A Quantitative Evaluation of the Relationships Between Growth Hormone Secretion and Delta Wave Electroencephalographic Activity During Normal Sleep and After Enrichment in Delta Waves

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Summary: The existence of a relationship between growth hormone (GH) release and slow-wave sleep (SWS), often studied in the past using conventional scoring of sleep stages, remains controversial. In the present study, this relationship was reevaluated by spectral analysis of the sleep electroencephalogram (EEG) and deconvolution analysis of the plasma GH concentrations during normal nocturnal sleep and after enrichment in SWS by means of ritanserin, a selective 5-HT_2 receptor antagonist. Eight healthy male subjects each participated in two randomized night studies after having received either a placebo or a 5-mg dose of ritanserin. They were subjected to 8 hours of polysomnography, including spectral analysis of the sleep EEG. Plasma GH levels were measured at 10-minute intervals. The mean delta absolute power and the mean GH secretory rates were significantly higher under ritanserin than under placebo for the first 3 hours after sleep onset (+24% and +29%, respectively). Their nocturnal profiles were significantly and positively correlated in all subjects (average $r = 0.710$ under placebo, 0.567 under ritanserin; $p < 0.0001$ in both cases). GH secretory pulses were found to be coincident with delta activity peaks in both directions. The amount of GH secreted during significant GH pulses was correlated with the amount of concomitant delta wave activity ($r = 0.803$ under placebo, $r = 0.764$ under ritanserin, $p < 0.0001$). Similarly, the amount of delta wave activity found during delta wave peaks was correlated with the amount of GH secreted concomitantly ($r = 0.715$ under placebo, $r = 0.723$ under ritanserin; $p < 0.0001$). These results demonstrate a close temporal and quantitative relationship between GH secretion and delta wave activity, which may be evidence of common stimulatory mechanisms. **Key Words:** Growth hormone secretion—Sleep—EEG spectral analysis—Delta wave activity.

Growth hormone (GH) is secreted by the anterior pituitary gland in a pulsatile fashion. Pulses are mainly controlled by the interaction between two hypothalamic peptides, growth hormone-releasing hormone (GHRH) that stimulates GH release and somatostatin (SRIF) that inhibits it. In previous studies, the 24-hour GH profile has been characterized by a sleep-dependent rhythm with a large secretory episode occurring just after sleep onset and temporally related to the first episode of slow-wave sleep (SWS) (1,2). This temporal relationship has been further examined and the authors did not exclude the possibility that the association between GH release and the first period of non-rapid eye movement (REM) sleep may be fortuitous (3,4).

The deconvolution procedure allows the calculation of hormone secretory rates from plasma concentrations using a mathematical model that removes the effects of hormonal distribution and degradation. Thus, this procedure provides a more accurate estimation of the secretory process than peripheral concentrations and allows for a more precise evaluation of the temporal concordance between hormonal secretion and other physiological events (5). Using this method combined with a high sampling frequency of 30 seconds for plasma GH, a detailed analysis showed that maximum GH release occurred within minutes after the onset of SWS (6). Similarly, a significant correlation was found be-

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tween the amount of the GH secreted in the SWS-associated pulses and the duration of SWS occurring during the pulse (7).

Spectral analysis of the sleep EEG appeared to be a useful tool in obtaining a quantitative analysis of the sleep EEG and a more detailed and dynamic description of the sleep processes than the traditional visual scoring of sleep stages (8,9). Jarret et al. (10), using this method, investigated the association between plasma GH concentrations and delta wave activity but were not able to find any significant quantitative relationship between the two parameters.

The aim of the present study was to reevaluate the quantitative relationship between GH secretion and delta wave activity, using both spectral analysis of the sleep EEG and deconvolution analysis. GH secretory rates were estimated from plasma levels for normal nocturnal sleep and after ritanserin intake. Ritanserin, a selective 5-HT_2 receptor antagonist, known to increase delta wave activity (11,12), was administered in order to test the robustness of the temporal relationship observed between GH secretion and delta wave activity and to examine whether this relationship is preserved in a quantitative manner when sleep is enriched in slow waves.

MATERIALS AND METHODS

Subjects and procedure

Eight healthy male subjects aged between 19 and 27 years and having a body mass index of 22.3 ± 0.7 kg/m^2 (mean ± SEM) participated in the study after medical examination. All had normal regular sleep–wake habits and none were taking medication in the 2 weeks prior to and during the study. Subjects with signs of underlying disease and smokers were excluded from the study. Before their final enlistment, they took part in an experimental session to familiarize themselves with the new environment and with catheter insertion. Informed written consent was obtained from all subjects and the experiment was approved by the local Ethics Committee.

The experiments were carried out in a sound-proof air-conditioned and electrically shielded sleep room. After an habituation night, all subjects underwent two randomized night studies with a 1-month interval between them. In a double-blind design, they received orally either placebo or a single dose of 5 mg ritanserin at 0900 hours, because a morning dose of ritanserin has been found to elicit the most pronounced increase in SWS in the subsequent night (13). During experimental nights, lights were switched off at 2300 hours and the subjects were awakened at 0700 hours. During the day preceding the experimental nights, sleeping was prevented by light activities. A controlled standard meal was given at 1900 hours.

Sleep analysis

Sleep recordings were performed using two electroencephalographic derivations (C3 or C4 versus A2 or A1 and Cz versus O1 or O2), one chin electromyographic derivation and one horizontal electrooculographic derivation (upper canthus of one eye versus lower canthus of the other eye). The recordings were visually scored at 30-second intervals using standardized criteria (14). For all-night spectral analysis, the electroencephalogram (EEG) signal (C3-O1 or C4-O2) was converted from analogue to digital with a sampling frequency of 128 Hz. Subsequently, spectra were computed for consecutive 2-second periods using a fast Fourier transformation (FFT) algorithm (15) and the values for 15 consecutive 2-second periods were averaged to yield power density values for 30-second periods. The spectral parameter studied was delta absolute power (0.5–3.5 Hz).

Blood sampling and plasma hormone measurements

A catheter was inserted at 1800 hours into an antecubital vein, which was kept patent with a heparin-containing solution. Blood was taken continuously from 2300 hours to 0700 hours using a peristaltic pump in an adjoining room. Samples were collected into Na_2 EDTA (1 mg/ml) tubes over 10-minute periods. They were immediately centrifuged at 4°C and the plasma stored at −25°C until analysis. A maximum of 100 ml blood was removed during the 8 hours.

Plasma GH concentrations were measured by radioimmunoassay (Sorin Biomedica, Saluggia, Italy). The detection limit was 0.3 ng/ml. The intra-assay coefficient of variation (CV) for duplicate samples was 15% for values less than 2 ng/ml, 8% between 2 and 5 ng/ml, and 5% for values above 5 ng/ml. All samples from one subject were measured in the same assay in order to avoid interassay variations.

Determination of GH secretory rates

The GH secretory rates were derived from the corresponding GH concentrations using a deconvolution procedure. A one-compartment model for hormone distribution and degradation was used with a subject-adjusted half-life lying between 21 and 18 minutes in order to minimize the number of false-negative secretory rates (7,16). The distribution volume was assumed to be 7% of the body weight (17). Statistical error

propagation of the uncertainty in plasma level measurements was taken into account in the determination of the standard deviation associated with each estimated secretory rate.

Cross-correlation analysis

The temporal relationship between the GH secretory rates and the delta absolute power profiles for the whole night was quantified using cross-correlation analysis (Box–Jenkins Time Series Analysis, BMDP Statistical Software). Cross-correlation coefficients were computed for lags [−2], [−1], [0], [+1], [+2] between the two chronological series, each lag corresponding to a 10-minute blood sampling interval. For negative lags, delta wave activity anticipates GH secretory rates, and conversely, for positive lags, GH secretory rates precede delta wave activity.

The individual correlation coefficients were averaged using Fisher's z transformation to yield an average estimate of the correlation (18). This average coefficient was computed following a χ^2 homogeneity test (19) on the individual transformed coefficients.

Pulse by pulse analysis

Determination of pulses of GH secretory rates

The individual profiles of GH secretory rates were analyzed and significant pulses were identified using a modification of the pulse detection algorithm ULTRA (20). An increase in the secretory rates was considered to be significant when the sum of the standard deviations associated with the successive estimated secretory rates was exceeded. Significant decreases were similarly identified. Thus, a secretory pulse was considered significant if both its increment and its decrement exceeded significant differences in secretory rates. For each significant pulse, the time of occurrence of the maximum level was determined and the amount of GH secreted during the pulse was calculated.

Determination of delta wave peaks

For quantification and characterization of the main delta absolute power peaks, the individual profiles were analyzed by a modification of the pulse analysis algorithm ULTRA. Taking into account the large interindividual variability in the levels of delta absolute power, the identification of the main peaks was achieved using a subject-adapted threshold for detection. This threshold was set at 20% of the maximum increment in delta absolute power observed in the subject. A peak was considered significant if both the increase and the decrease exceeded this threshold. For each significant peak, the time of occurrence of the maximum was determined and the amount of delta power was calculated by means of the area under the curve corresponding to the peak.

Coincidence analysis

Pulses of GH and peaks of delta waves were considered to be coincident when they occurred in a time-window of five sampling points ([−2], [−1], [0], [+1], [+2]). The probability associated with the observed coincidence was assessed using the hypergeometric probability density function (21).

Estimation of the quantitative relationship between GH secretion and delta waves

The quantitative relationship between GH secretion and delta wave activity was estimated using a linear correlation between the amount of GH secreted during each significant pulse and the amount of delta absolute power corresponding to this GH pulse. In order to estimate the overall relationship for all of the subjects, all individual data were pooled. In an attempt to make all scores comparable for all subjects, each score was removed from an estimate of the individual effect to produce a set of aligned observations and a linear correlation coefficient was calculated from aligned data (22). Conversely, a linear correlation was estimated between the amount of delta waves during each significant delta wave peak and the corresponding amount of GH secreted.

The nonparametric Wilcoxon matched-pairs signed-ranks test was used to determine statistical significance of differences between the mean nocturnal GH secretory rates and delta absolute power levels obtained under placebo vs. ritanserin. Group values are given as the mean ± SE.

RESULTS

Mean profiles of delta absolute power and GH secretory rates

The mean profiles of delta absolute power and GH secretory rates under placebo and under ritanserin are represented in Fig. 1. They indicate that in both conditions the highest amount of GH is secreted during the first 3 hours following sleep onset (85% of the total amount secreted during the sleep period under placebo and 90% under ritanserin), a period for which delta relative power was also highest. As expected, a significant 22% enhancement of the mean overnight delta absolute power was observed under ritanserin (41.9 ± 8.7 μV^2 vs. 34.3 ± 9.3 μV^2, $p = 0.0078$). This increase

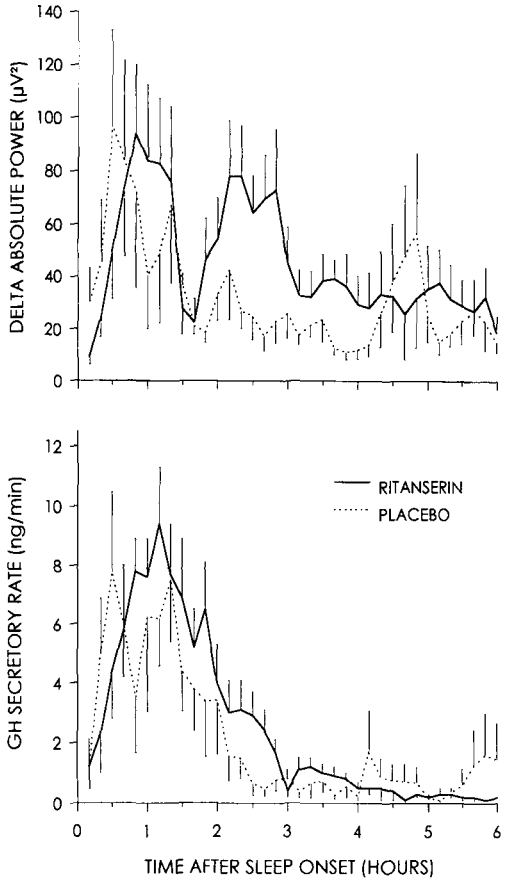

FIG. 1. Mean profiles (± SEM) of delta absolute power (top) and GH secretory rates during placebo and ritanserin nights. The delta absolute power and the GH secretory rates are significantly higher under ritanserin than under placebo for the first 3 hours after sleep onset (+24% and +29%, respectively).

was 24% for the first 3 hours after sleep onset (44.3 ± 11.7 μV^2 vs. 54.6 ± 12.3 μV^2). Mean overnight GH secretory rates were not statistically different in the two experimental conditions. However, during the first 3 hours following sleep onset, a significant 29% increase in the mean GH secretory rates was observed during ritanserin nights (3.59 ± 0.61 μV^2 vs. 4.62 ± 0.74 μV^2, $p = 0.0469$).

Cross-correlation analysis

In all subjects, GH secretory rates were significantly and positively correlated with delta wave activity, so that a rise in delta wave activity was temporally associated with an increase of GH secretion. Cross-correlation coefficients between GH secretory rates and delta absolute power profiles were highest for lags [−1], [0], and [+1], indicating that the two chronological series were for the most part concomitant (11 out of 16), but that sometimes GH secretion preceded delta wave activity by 10 minutes, or conversely that the delta waves anticipated GH secretion by 10 minutes (Table 1). In both conditions, the χ^2 test of homogeneity revealed that the individual cross-correlation coefficients were homogeneous and the average correlation coefficient was found to be highly significant in both cases ($r = 0.710$ under placebo vs. $r = 0.567$ under ritanserin; $p < 0.0001$). This average coefficient was not significantly different in the two conditions ($p = 0.10$). Figure 2 illustrates individual profiles of GH secretory rates and delta absolute power for two representative subjects under the two conditions, which offers evidence of a close overall relationship between the two parameters during the night.

Coincidence analysis

GH secretory pulses were found to be significantly coincident with delta activity peaks in seven of the eight subjects studied, as well in placebo as in ritanserin nights (Table 2). This proportion reached significance by the binomial test ($p = 0.0352$, one-tailed), providing evidence for a consistent temporal relationship between the two parameters.

TABLE 1. *Cross-correlations between GH secretory rates and delta absolute power profiles*

	Placebo				Ritanserin			
Subjects	*r*	Lag	n	p	*r*	Lag	n	p
1	0.835	0	44	0.0000	0.556	0	46	0.0001
2	0.717	0	39	0.0000	0.714	0	41	0.0000
3	0.747	0	46	0.0000	0.623	0	44	0.0000
4	0.806	1	47	0.0000	0.363	0	46	0.0132
5	0.595	−1	44	0.0000	0.756	0	47	0.0000
6	0.718	0	44	0.0000	0.542	−1	46	0.0001
7	0.605	0	46	0.0000	0.468	−1	46	0.0010
8	0.482	1	35	0.0034	0.413	0	46	0.0043
χ^2		14.05				13.54		
Average *r*		0.710				0.567		
p		$p < 0.0001$				$p < 0.0001$		

n = Number of 10-minute plasma samples after sleep onset.

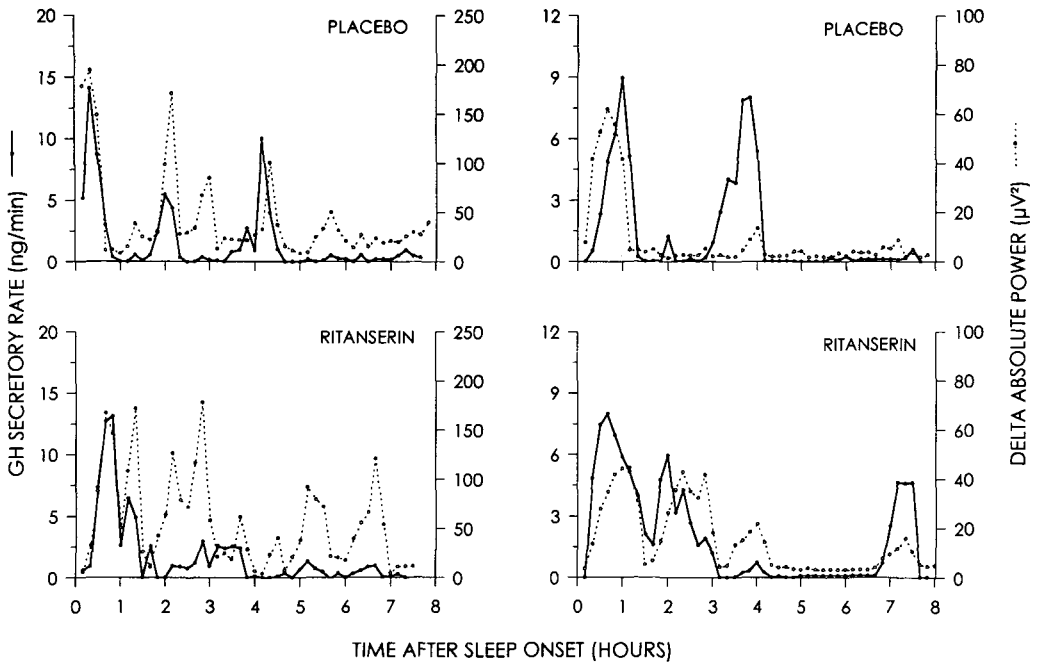

FIG. 2. Concomitant delta absolute power and GH secretory profiles in two representative subjects during placebo and ritanserin nights. In both experimental conditions fluctuations of GH secretory rates are closely related to fluctuations of delta wave activity.

Quantitative relationship between GH secretory rates and delta absolute power

In order to determine whether GH secretory activity and the delta absolute power are quantitatively linked, a two-step analysis was performed.

A total of 38 significant GH secretory pulses were observed during placebo nights and 33 significant GH pulses during ritanserin nights ($p = 0.26$, NS). For each significant pulse, the amount of GH released was calculated and correlated with the concomitant amount of delta waves for each subject. This analysis was performed on aggregate data individually aligned. Figure 3 illustrates the significant linear correlation found under placebo and under ritanserin ($r = 0.803$, $p < 0.0001$; $r = 0.764$, $p < 0.0001$).

A total of 32 significant peaks of delta waves were found in the placebo condition and 43 in the ritanserin condition ($p = 0.40$, nonsignificant difference). The amount of delta absolute power determined for each significant peak was found to be significantly correlated with the concomitant amount of GH secreted ($r = 0.715$, $p < 0.0001$ under placebo; $r = 0.723$, $p < 0.0001$ under ritanserin) (Fig. 4).

DISCUSSION

The results of the present study show that during normal night sleep, a temporal link exists between the GH secretory profile and brain EEG activity expressed by delta wave activity. A quantitative relationship between the amount of GH secreted and the concomitant delta wave activity could be statistically

TABLE 2. *Coincidence analysis between significant delta absolute power and GH secretary rate pulses*

	Placebo				Ritanserin			
	Number of pulses				Number of pulses			
Subjects	Delta	GH	Coincidence	p	Delta	GH	Coincidence	p
1	5	4	4	0.0000	6	3	3	0.0013
2	3	2	1	0.1457	4	4	3	0.0015
3	5	5	4	0.0001	7	5	5	0.0000
4	4	7	4	0.0002	7	4	3	0.0084
5	2	4	2	0.0058	5	4	3	0.0024
6	2	4	2	0.0061	6	5	2	0.1081
7	6	6	3	0.0199	5	4	3	0.0024
8	5	6	3	0.0250	3	4	3	0.0003

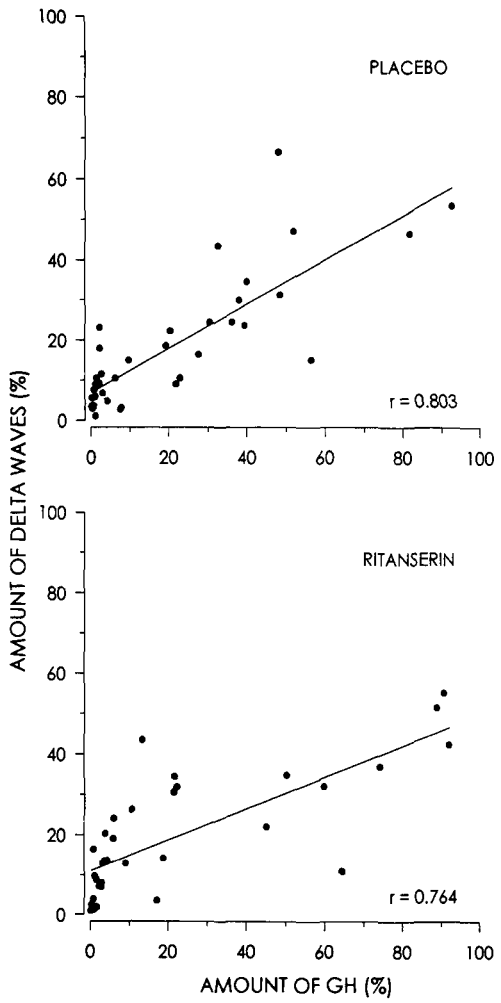

FIG. 3. Relationship between the amount of GH secreted during each significant GH pulse and the amount of concomitant delta waves. Data are represented in percentage (%) of the total amount of each variable calculated for the whole night following sleep onset. The correlation coefficients are highly significant in both experimental conditions ($p < 0.0001$).

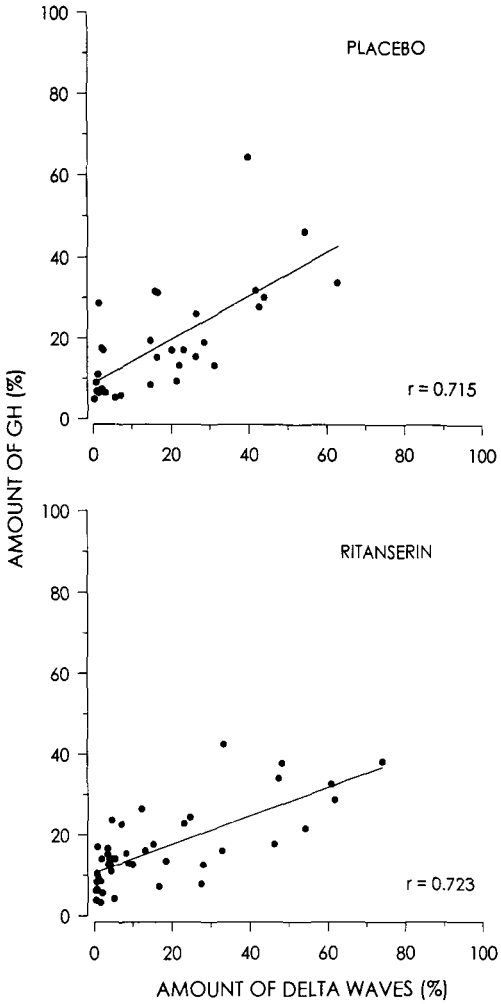

FIG. 4. Relationship between the amount of delta waves found during the main delta wave peaks and the amount of GH secreted concomitantly. Data are represented in percentage (%) of the total amount of each variable calculated for the whole night following sleep onset. The correlation coefficients are highly significant in both experimental conditions ($p < 0.0001$).

assessed. Also when the amount of delta sleep was enhanced by previous ritanserin intake, GH secretory rates increased in a manner similar to delta wave activity and thus the two parameters remained quantitatively associated.

The discrepancies between the results of previous reports, concluding either a rather fortuitous (3,4,10) or a significant relationship between SWS and GH release (1,2,7), may be explained by the different methodologies used. Most of these previous studies relied on traditional sleep stage analysis, which tends to obscure the fact that sleep is a continuous process. In order to make possible a more refined exploration of the dynamic aspects of sleep, our results are based on spectral analysis of the sleep EEG, and delta wave activity was taken as a sensitive indicator of SWS. In addition, the temporal organization of the GH secretory profile during sleep was precisely characterized by deconvolution of the peripheral plasma concentrations, an approach that allowed synchrony between GH secretion and delta wave activity to be estimated accurately and also allowed quantification of the amount of GH secreted.

The correlation coefficients found in this study indicate the strength of the temporal relationship between the two parameters. Cross-correlation analysis assesses the tendency for two time series to covary in the same or opposite directions simultaneously either with a positive or negative lag and thus estimates the overall coordinate behavior of the two series. This analysis is not adapted to estimating temporal links between discrete events occurring in the series such as

the pulses of GH secretion and the peaks of delta wave activity. Indeed, a large concomitant pulse in the two series strongly influences the correlation, masking the effect of nonsynchronized small peaks even if they are more numerous.

Taking into account the limitations of this analytical method, the association of the two parameters was further assessed by two other analyses. Copulsatility was demonstrated by a hypergeometric probability calculation, which indicates that GH secretory rates and delta absolute power peaks present a high degree of nonrandom coincidence, meaning that they are temporally associated in a nonrandom manner in any given subject.

In addition a significant linear correlation was found to exist between the amount of GH secreted during each significant GH pulse and the amount of delta waves recorded concomitantly. This indicates that a quantitative relationship exists between the two series. Conversely, the amount of delta waves, calculated during each significant delta relative power peak, is also correlated with the amount of GH released concomitantly, which could mean that delta wave activity modulates the GH secretory rates.

However, this relationship, albeit quantitative, is not necessarily causal because it has been shown that GH secretion can be pharmacologically dissociated from SWS. Flurazepam markedly reduces SWS but does not alter the sleep-related GH secretion (23). A virtual abolition of GH secretion was observed after the administration of methscopolamine, while having no effect on SWS (24). More recently, it has been shown that the administration of scopolamine shifted GH secretion into the late portion of the night even though SWS distribution was not affected (25).

Results of other studies would further indicate a closer link between the two parameters. Thus an administration of GHRH has been found to produce a concomitant increase in plasma GH concentrations and in SWS (26), although sleep-promoting effects of GHRH, especially for SWS, have been described as depending on time of administration of the peptide (27). Another study concluded with a GHRH-induced increase in GH release associated with an increase in the theta band (4–10 Hz), but not in the delta band, of the sleep EEG (28). Moreover, a GHRH antagonist produced an increase in non-REM sleep latency in rats and a decrease in its duration concomitant with an inhibition of GH secretion (29). On the other hand, a decrease in GH concentrations and in SWS was observed following the administration of corticotropin releasing hormone (CRH) (30). These latter results suggest that regulatory mechanisms of GH secretion and SWS may be under common central control. In a similar vein, a model for GH secretion has been worked out by Hartman et al. (31). This model is based on the hypothesis that the GH secretory pulses result from the interaction of multiple pulses of hypothalamic GHRH secretion stimulating the pituitary gland during diminished somatostatin secretion. Thereby, the linkage between GH and SWS may involve somatostatin withdrawal during SWS (32,33).

Few studies have investigated hormonal responses to an acute administration of ritanserin in man and results are not consistent. Recent results showed that GH levels are not affected by a single 10-mg dose of ritanserin (34) as well as by a 30-mg dose (35), whereas others found that a 10-mg ritanserin dose produced a slight reduction of GH concentrations (36). Thus, the effect of ritanserin on GH is as yet unclear. In our study, ritanserin was administered for its sleep-deepening properties because it is well known that this drug enhances the amount of SWS in man (11,12) as well as in animals (37–39). However REM sleep latency and duration were not affected by ritanserin or seganserin, as observed after sleep deprivation, and it remained questionable if ritanserin promotes a physiological type of SWS (39,40). Indeed, we observed an increase in delta wave activity, which mainly occurred during the first 3 hours following sleep onset and was concomitant and proportionally accompanied by an increase in GH levels. A low dose of ritanserin was used in this study, and taking into account the long delay between its administration and the onset of the hormonal measurements, a direct effect of ritanserin on GH is unlikely. Thus the GH increase observed in the first 3 hours following sleep onset may be essentially attributable to delta sleep-related effects, which strengthens the results obtained in placebo conditions. These findings suggest that the regulatory mechanisms involved in the control of delta wave activity may stimulate GH secretion so that the two parameters could be related in a significant quantitative manner.

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